Claims

1. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a second ligand having a second biodistribution different from that of the first ligand, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand.

2. The composition according to claim 1, further comprising a physiologically acceptable excipient.

3. The composition according to claim 2, wherein the multispecific ligand comprises a bispecific antibody.

4. The composition according to claim 3, wherein the affinity of said first ligand binding moiety for the first ligand is higher than the affinity of the second ligand binding moiety for the second ligand and wherein the biodistribution of the multispecific ligand favours the first ligand.

5. The composition according to claim 4, wherein the first and second ligands have overlapping biodistributions.

6. The composition according to claim 5, wherein the first ligand is present on a first target cell population and wherein said second ligand is present on a second target cell population comprising the first target cell population and wherein the biodistribution of the multispecific ligand favours the first target cell population.

7. The composition according to claim 1 or 3, wherein said first ligand is a cell surface marker associated with one or more specific cell populations, infectious or parasitic agents, diseased cells, or disease-associated cells.

8. The composition according to claim 7, wherein said marker is an antigen.

9. The composition according to claim 7, wherein said marker is an epitope.

10. The composition according to claim 7, wherein said marker is a CD marker.

11. The composition according to claim 10, wherein said marker is CD4.

12. The composition according to claim 7, wherein said marker is specifically associated with a cancer cell or pre-cancerous cell.

13. The composition according to claim 11 or 12, wherein said second ligand is a CCR5 or CXCR4 receptor.

14. The composition according to claim 7, wherein said marker is associated with an immune cell that is susceptible to viral infection.

15. The composition according to claim 7, wherein said marker is specifically associated with an autoimmune disorder or rheumatic disease.

16. The composition according to claim 7, wherein said marker is associated with a specific tissue type.

17. The composition according to claim 7, wherein said marker is associated with a specific organ.

18. The composition according to claim 7, wherein said marker is associated with a cell or tissue of specific origin or class.

19. The composition according to claim 7, wherein said marker is an MHC-peptide complex.

20. The composition according to claim 7, wherein said marker is a cell surface immunoglobulin.

21. The composition according to claim 6 or 7, wherein said second ligand is a cell surface receptor, a family of cell surface receptors or one or more particular cell surface receptor family members.

22. The composition according to claim 21, wherein said second ligand is a cell surface receptor.

23. The composition according to claim 22, wherein said second ligand is a marker associated with a lymphatic endothelial cell.

24. The composition according to claim 22, wherein said second ligand is a cell surface receptor is selected from the group consisting of tyrosine kinase type receptors, serine kinase type receptors, heterotrimeric G-protein coupled receptors, receptors bound to tyrosine kinase, TNF family receptors, notch family receptors, guanylate cyclase types, tyrosine phosphatase types, decoy receptors, and adhesion receptors.

25. The composition according to claim 22, wherein said second ligand is an IL-8 receptor, a CCR7 receptor, a FAS receptor, or a CXCR4 receptor.

26. The composition according to claim 22, wherein said receptor requires cross-linking for biological activity.

27. The composition according to claim 22, wherein binding of said second ligand binding moiety to said cell surface receptor blocks said receptor.

28. The composition d according to claim 22, wherein binding of said second ligand binding moiety to said cell surface receptor activates said receptor.

29. The composition according to claim 22, wherein said cell surface receptor initiates a signal transduction and wherein binding of said second ligand binding moiety to said cell surface receptor effects a signal transduction.

30. The composition according to claim 6, 7 or 22, wherein said antibody comprises a first VH which specifically recognizes said first ligand and a second VH which specifically recognizes said second ligand.

31. The composition according to claim 30, wherein at least one of said first and second VHs require a VL for binding to its ligand.

32. The composition according to claim 31, comprising a first VL in functional association with said first VH and a second VL in functional association with said second VH and wherein both said first and second functional associations are required for binding to the first and second ligands, respectively, and wherein said first and second VLs are the same or functionally interchangeable.

33. The composition according to claim 31 or 32, wherein said antibody is a four chain antibody.

34. The composition according to claim 33, wherein said antibody is a minibody or antibody lacking a CH3 domain.

35. The composition according to claim 33, wherein said antibody is a diabody.

36. The composition according to claim 31 or 32, wherein said antibody lacks antibody light chains.

37. The composition according to claim 32, wherein said antibody comprises a pair of disulfide linked heavy chains or heavy chain portions each comprising at least a VH region, a hinge region and at least a portion of an Fc region at the carboxy terminus of the hinge region.

38. The composition according to claim 37, wherein said bispecific antibody comprises a pair of VHs linked through a flexible linker.

39. The composition according to claim 4, 6, 7 or 22 wherein the affinity of the first ligand binding moiety for the first ligand is at least approximately, one, two, three, four, five, six, seven or eight orders of magnitude greater than the affinity of said second ligand binding moiety for the second ligand.

40. A composition comprising a multispecific ligand comprising a first ligand binding moiety which specifically binds with a pre-selected first affinity to a first ligand having a first biodistribution and a second ligand binding moiety which

specifically binds with a pre-selected affinity to a second ligand having a second biodistribution, and wherein the affinity of first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand.

41. The composition according to claim 40, further comprising a physiologically acceptable excipient.

42. The composition according to claim 1 or 40, wherein the biodistributions of said first and second ligands overlap and wherein the affinities of the first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand in favour of a target cell population on which both first and second biodistributions occur relative to one or more non-target cell populations.

43. The composition according to claim 42, wherein the affinities of said first and second ligand binding moieties are both selected to limit their individual ability to bind to the first and second ligands, respectively, and wherein their combined functional affinity biases the distribution of the multispecific ligand towards said target cell population.

44. The composition according to claim 42, wherein the affinity of first ligand binding moiety for the first ligand is at least, approximately, one, two, three, four, five, six, seven or eight orders of magnitude greater than the affinity of the second ligand binding moiety for the second ligand.

45. The composition according to claim 42 or 44, wherein first and second ligands are recognized contemporaneously by the first and second ligand binding moieties.

46. A composition comprising a multispecific ligand which specifically binds to a target ligand on a selected sub-population of a heterogeneous cell population bearing the target ligand, the multispecific ligand comprising a first ligand binding moiety which specifically binds to a cell sub-population associated ligand and a second ligand binding moiety which binds to the target ligand, said first ligand binding moiety having an affinity for the sub-population associated ligand that is higher than the affinity of the second ligand binding moiety for the target ligand.

47. The composition according to claim 46, further comprising a physiologically acceptable excipient.

48. The composition according to claim 46, wherein the affinity of said first ligand binding moiety for the cell sub-population associated ligand is

approximately, one, two, three, four, five, six, seven or eight orders of magnitude greater than the affinity of said second ligand binding moiety for said target ligand.

49. The composition according to claim 48, wherein said target ligand is a receptor.

50. The composition according to claim 46, wherein at least one of said first or second ligand binding moieties comprises an antibody heavy chain or functional portion(s) thereof including a VH or fragment thereof and an antibody light chain or functional portion(s) thereof including a VH or fragment thereof.

51. A composition comprising an antibody which specifically binds to an epitope on a ligand wherein said ligand exerts a biologic effect by binding to a target site on a target ligand through an affinity for said target ligand, said epitope being proximal to the binding site of said ligand for the target ligand, such that the antibody reduces but does not prevent the affinity of the ligand for its target ligand.

52. The composition according to claim 51, further comprising a physiologically acceptable excipient.

53. A composition comprising a multispecific ligand comprising a first ligand binding moiety which specifically binds to a lymphatic endothelial cell associated marker and a second moiety comprising a therapeutic moiety.

54. The composition according to claim 53, further comprising a physiologically acceptable excipient.

55. The composition according to claim 53, wherein the therapeutic moiety provides an immune function.

56. The composition according to claim 53, wherein the marker is selected to limit the ability of said endothelial cell to internalize said multispecific ligand.

57. The composition according to claim 53, wherein said first portion is an antibody.

58. The composition according to claim 54, wherein said second portion moiety binds to a target ligand.

59. The composition according to claim 53, wherein said therapeutic moiety comprises an antibody moiety.

60. The composition according to claim 53, wherein said ligand is selected from the group consisting of CCR5, CTLA-4, LFA-1, ICAM-1, CD2, CD3, CD4, CD22, CD40, CD44; CD80, CD86, CD134 and CD154.

61. The composition according to claim 53, wherein said first portion binds to LYVE-1 or podoplantin.

62. The composition according to claim 53, wherein said second portion comprises an anti-idiotypic antibody.

63. The composition according to claim 62, wherein said anti-idiotypic antibody binds to an autoimmune antibody.

64. The composition according to claim 63, wherein said anti-idiotypic antibody mimics a cell surface expressed tumour antigen.

65. The composition according to claim 53, wherein said second portion binds to a diseased cell.

66. The composition according to claim 65, wherein said diseased cell is a cancer cell.

67. The composition according to claim 53, wherein said second portion binds to an infectious agent or parasite.

68. The composition according to claim 67, wherein said diseased cell is a virally infected cell.

69. The composition according to claim 53, wherein said second portion binds to a cell of the immune system.

70. The composition according to claim 69, wherein immune cell is associated with an autoimmune reaction.

71. The composition according to claim 69, wherein said immune cell is a CCR5-expressing cell.

72. The composition according to claim 64 or 69, wherein said second portion binds with greater functional affinity to its target ligand than said first portion binds to its target ligand.

73. The composition according to claim 64 or 69, wherein said second portion binds with greater affinity to its target ligand than said first portion binds to its target ligand.

74. The composition according to claim 53, wherein said second portion binds with greater avidity to its target ligand than said first portion binds to its target ligand.

75. The composition according to claim 53, wherein said second portion comprises an internalizing antibody and a cytotoxic component.

76. The composition according to claim 53, wherein said multispecific ligand is a bispecific antibody having a monovalent first portion and a monovalent second portion.

77. The composition according to claim 53, wherein said multispecific ligand is a bispecific antibody having a divalent first portion and a divalent second portion.

78. The composition according to claim 53, wherein said multispecific ligand is a trispecific antibody having a monovalent first portion and a second portion comprising a divalent immune function exerting moiety which binds to one or more target ligands on a target diseased cell or immune cell and a monovalent anti-CD3 or anti-CD28 antibody.

79. The composition according to claim 53, wherein said multispecific ligand is a trivalent trispecific antibody having a monovalent first portion and a second portion comprising a monovalent immune function exerting moiety which binds to a target ligand on a target diseased or immune cell and a monovalent anti-CD3 or anti-CD28 antibody.

80. The composition according to claim 53, wherein said multispecific ligand is a trivalent trispecific antibody having a monovalent first portion and a second portion comprising a divalent immune function exerting moiety which binds to a target ligand on a target diseased or immune cell.

81. The composition according to claim 53, wherein said second portion comprise a cytokine component.

82. The composition according to claim 53, wherein said second portion comprises a cytotoxic component.

83. The composition according to claim 53, wherein said second portion comprises a ligand capable of binding to T cells.

84. The composition according to claim 83, wherein said ligand is an antibody which binds to T cells.

85. The composition according to claim 53, wherein said second portion comprises an anti-CD3 antibody or anti-CD28 antibody.

86. The composition according to claim 53, wherein second portion is a cytokine component.

87. The composition according to claim 53, wherein second portion is an anti-CD3 antibody or an anti-CD28 antibody.

88. The composition according to claim 53, wherein said second portion further comprises one or more components selected from the group consisting of a cytokine component, a cytotoxic component and an anti-CD3/CD28 component.

89. A composition comprising an immunocytokine having an anti-idiotypic antibody component which recognizes the paratope of an antibody which binds to a lymphatic vessel associated ligand and a cytokine component.

90. The composition according to claim 89, wherein the cytokine component is fused with or conjugated to the lymphatic vessel associated ligand.

91. An immunocytokine as claimed in claim 89, wherein said cytokine component comprises IL-2 or a functional fragment thereof and/or IL-12 or a functional fragment thereof.

92. An immunocytokine as claimed in claim 89, wherein said cytokine component comprises TNF-α or a functional fragment thereof.

93. A composition comprising a bispecific antibody having an anti-idiotypic antibody component which recognizes the paratope of an antibody which binds specifically to a lymphatic vessel associated ligand and an anti-CD3 antibody or an anti-CD28 antibody component.

94. The composition according to claim 90 or 91, wherein said anti-idiotypic antibody component has a lower functional affinity for the paratope of the antibody which binds specifically to the lymphatic vessel associated ligand than the latter antibody has for the lymphatic vessel associated ligand.

95. A composition comprising a bispecific ligand comprising a first ligand which binds to a first target ligand and a second ligand which binds to a second target ligand, and wherein the affinity of said first ligand is selected to enable binding to the first target ligand independently of the ability of said second ligand to bind to the second target ligand and wherein the affinity of said second ligand is selected to substantially reduce the probability of its binding to the second target ligand without the first ligand binding first or substantially contemporaneously to the first target ligand.

96. A composition comprising a bispecific antibody comprising a first antibody component which binds to a first target ligand and a second antibody component which binds to a second target ligand, and wherein the affinity or avidity or both the affinity and avidity of said first antibody component are selected to enable binding to the first target ligand independently of the ability of said second antibody component to bind to the second target ligand and wherein the avidity or affinity or both the affinity and avidity of said second ligand are selected to substantially reduce the probability of its binding to the second target ligand without the first ligand binding first or substantially contemporaneously to the first target ligand.

97. A composition comprising a multispecific ligand comprising a first moiety which binds to a first target ligand and a second moiety which binds to a second target ligand, and wherein the affinity or avidity or both the affinity and avidity of said first moiety are selected to enable the first moiety to bind to the first target ligand independently of the ability of said second moiety to bind to the second target ligand and wherein the avidity or affinity or both the affinity and avidity of said second moiety are selected to substantially reduce the probability of its binding to the second target ligand without the first moiety, first or substantially contemporaneously, binding to the first target ligand.

98. The composition according to claim 97, wherein both moieties bind to different target ligands on the same cell.

99. A composition comprising a multispecific ligand comprising a first moiety which binds to a first target ligand and a second moiety which binds to a second target ligand, and wherein the affinity or avidity or both the affinity and avidity of said first moiety are selected to enable the first moiety to bind to the first target ligand independently of the ability of said second moiety to bind to the second target ligand and wherein the avidity or affinity or both the affinity and avidity of said second moiety are selected to substantially reduce the probability of either moiety binding for a sufficient duration or series of durations to its respective target ligand to accomplish a therapeutic function without the other moiety, first or substantially contemporaneously, binding to its respective target ligand.

100. The composition according to claim 99, wherein both moieties bind to different target ligands on the same cell.

101. A composition comprising a multispecific ligand comprising a first moiety which binds to a first target ligand and a second moiety which binds to a second target ligand, and wherein the affinity or avidity or both the affinity and avidity of said first moiety are selected to enable the first moiety to bind to the first target ligand independently of the ability of said second moiety to bind to the second target ligand and wherein the avidity or affinity or both the affinity and avidity of said second moiety are selected to enable the second moiety to bind to the second entity in preference to the first moiety binding to the first entity when both first and second moieties are substantially contemporaneously bound to the respective first and second entities.

102. The composition according to claim 101, wherein the first moiety comprises at least one antibody component which binds to a first cell and the

second moiety comprises at least one antibody component which binds to a second different cell.

103. A composition comprising a multispecific ligand comprising a first moiety which binds to a first target ligand and a second moiety which binds to a second target ligand, and wherein the affinity or avidity or both the affinity and avidity of said first moiety are selected to enable the first moiety to bind to the first target ligand independently of the ability of said second moiety to bind to the second target ligand and wherein the avidity or affinity or both the affinity and avidity of said second moiety to bind to the second target ligand and wherein the avidity or affinity or both the affinity and avidity of said first moiety are selected to enable the first moiety to bind to the first entity in preference to the second moiety binding to the second entity when both first and second moieties are substantially contemporaneously bound to the respective first and second entities, and wherein the avidity or affinity or both the affinity and avidity of said second moiety are selected to enable the third target ligand to bind to the second entity in preference to the second moiety binding to the second entity when both said third target ligand and the second moiety are substantially contemporaneously bound to the second entity.

104. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds with a pre-selected first affinity to at least a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds with a pre-selected affinity to at least a second ligand having a second biodistribution, and wherein the affinity of first and second ligand binding moieties are selected to bias the biodistribution of the multispecific ligand in favour of a selected location of one or both of the ligands.

105. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a second ligand having a second biodistribution, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand, and wherein the affinity of the first ligand binding moiety for the first ligand is at least, approximately, one order of magnitude greater than that of the second ligand binding moiety for the second ligand.

106. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a

second ligand having a second biodistribution, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand, and wherein the affinity of the first ligand binding moiety for the first ligand is at least, approximately, two orders of magnitude greater than that of the second ligand binding moiety for the second ligand.

107. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a second ligand having a second biodistribution, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand, and wherein the affinity of the first ligand binding moiety for the first ligand is at least, approximately, three orders of magnitude greater than that of the second ligand binding moiety for the second ligand.

108. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a second ligand having a second biodistribution, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand, and wherein the affinity of the first ligand binding moiety for the first ligand is at least, approximately, four orders of magnitude greater than that of the second ligand binding moiety for the second ligand.

109. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a second ligand having a second biodistribution, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand, and wherein the affinity of the first ligand binding moiety for the first ligand is at least, approximately, five orders of magnitude greater than that of the second ligand binding moiety for the second ligand.

110. A composition comprising a multispecific ligand comprising at least a first ligand binding moiety which specifically binds to a first ligand having a first biodistribution and a second ligand binding moiety which specifically binds to a

second ligand having a second biodistribution, and wherein the affinity of the first and second ligand binding moieties are different and selected to bias the biodistribution of the multispecific ligand, and wherein the affinity of the first ligand binding moiety for the first ligand is at least, approximately, six orders of magnitude greater than that of the second ligand binding moiety for the second ligand.

111. A composition according to any one of claims 105 to 110, wherein the biodistributions of said first and second ligands comprise a target population of cells and at least one non-target population of cells and wherein said first and second ligands are present only on said target population and wherein the biodistribution of the multispecific ligand is biased in favor of the target population of cells.

112. A composition according to claim 111, wherein said multispecific ligand is adapted to bind to two ligands on the same cell.

113. A composition according to claim 112, wherein said multispecific ligand comprises at least two full length heavy chains or heavy chain fragments having differing specificities, or is chosen from a $F(ab')_2$, a minibody, a diabody, a four chain immunoglobulin having a truncated Fc portion, a tetravalent antibody having a four chain framework and a divalent Fab.

114. A host cell or cell free expression medium comprising one or more polynucleotides, said one or more polynucleotides comprising one or more DNA sequences, said one or more DNA sequences comprising one or more polypeptides which are sufficient to constitute a multispecific ligand as defined in any of the preceding claims

115. A kit comprising one or more polynucleotides, said one or more polynucleotides comprising one or more DNA sequences, said one or more DNA sequences encoding one or more polypeptides which are sufficient to constitute a multispecific ligand as defined in any of the preceding claims.

116. A liquid medium comprising comprising one or more polypeptides which are sufficient to constitute a multispecific ligand as defined in any of the preceding claims.

117. A liquid medium comprising one or more host cells, said one or more host cells comprising one or more polynucleotides, said one or more polynucleotides comprising one or more DNA sequences, said one or more DNA sequences encoding one or more polypeptides which are sufficient to constitute a multispecific ligand as defined in any of the preceding claims.

118. A substantially isolated polynucelotide comprising one or more DNA sequences, said , said one or more DNA sequences encoding one or more polypeptides which are sufficient to constitute a multispecific ligand as defined in any of the preceding claims

119. A substantially isolated polynuceotide comprising a DNA sequence encoding a polypeptide portion of a second ligand binding moiety as defined in any of the preceding claims, said polypeptide portion comprising a VH or VL, said second ligand binding moiety having a low affinity for said second ligand.

120. A substantially isolated polynucleotide according to paragraph 119, wherein said polynucleotide is a substantially isolated expression or cloning vector.

121. A method of making a multispecific ligand as defined in any of the preceding paragraphs comprising expressing at least one polynucleotide as defined in claim 115, 118, 119 or 120.

122. A pharmaceutical composition comprising a multispecific ligand as defined in any of the preceding claims and a pharmaceutically acceptable excipient.

123. A therapeutic composition comprising a multispecific ligand as defined in any of the preceding paragraphs and a pharmaceutically acceptable excipient.

124. A method of treating a disease in a mammal comprising administering a therapeutically effective amount of a multispecific ligand according to any of the preceding claims.

125. A kit comprising a plurality of different multispecific ligands as defined herein.

126. A composition according to claim 1, 111 or 112, wherein at least one of said first and second ligand binding moieties comprises human sequences.

127. A composition according to claim 1 or 111, wherein at least one of said first and second ligand binding moieties comprises human framework sequences.

128. A combinatorial library comprising a diverse population of multispecific ligands according to claim 1, 111 or 112, chararacterized by members of said population having a diversity of affinities for at least one of said first and second ligands.

129. A diverse population of nucleic acids which encode a combinatorial library as defined in claim 28.

130. A multispecific ligand according to any of claims 1 to 10, wherein said first ligand is on a cell population which circulates in the blood.